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55. The method of claim 53, wherein said *Shigella* is further inactivated.-- D

REMARKS

With entry of this amendment, claims 45-55 are under examination. The claims have been rewritten to more particularly specify preferred embodiments of the invention. Support for the new claims can be found in the originally filed claims and throughout the specification. No new matter has been added. Reconsideration is requested.

Claims 28 and 31-33 were rejected under 35 USC § 102(e) as being anticipated by Curtiss,III (U.S. Pat. No. 5,672,345). Claims 29, 30 and 44 were rejected under 35 USC § 103(a) as being obvious in view of Curtiss,III. To the extent to which these rejections might be considered applicable to the presently pending claims, they are traversed for the following reasons.

The Curtiss patent discloses a vaccine for immunization. Although exogenous DNA is delivered to animal cells, it is not a part of a delivery system such as that of the present invention, i.e. bacteria which lyse after entry into cells and deliver expressible DNA. The method of Curtiss delivers an antigen, for the purpose of stimulating an immune response in the animal. In the method of Curtiss, the DNA is expressed in the bacterial cell; in the present invention, it is expressed in the target cell. It is submitted that the presently

pending claims are neither anticipated by nor obvious from Curtiss.

Claims 28-33 and 44 were rejected under 35 USC § 112, first paragraph as not being enabled. It is the Examiner's position that the specification is enabling for attenuation of *Shigella* strains by inactivation of *asd*, but not for other mutating factors which will result in lysis of *Shigella*, or of other bacteria as broadly claimed. It is also the Examiner's position that cells other than mammalian cells have not been taught and are not enabled. To the extent to which these rejections might be considered to be applicable to the presently pending claims, they are traversed for the following reasons.

The Examiner's attention is respectfully directed to the Rule 132 Declarations of Dr. Donata Sizemore and Dr. Arthur Branstrom, filed herewith, which relate to the discussion following.

The Examiner has acknowledged that *Shigella asd* mutants are able to invade mammalian host cells, disrupt the endocytic vesicle, and subsequently die by lysis, thereby releasing their nucleic acid into the host cell for expression. As demonstrated in the Rule 132 Declarations filed herewith, this process can be accomplished using other bacteria, other mutations, and other host cells than are contained in the detailed examples of the specification.

1) <u>Enablement for other mutations</u>

The Declaration of Dr. Arthur Branstrom provides evidence which demonstrates that other methods of producing such mutated bacteria are known and would function according to the invention. Means of producing such mutated bacteria include:

a. Bacterial autolysins (Genbank seq. #D17366)

b. Phage mediated lysis

c. Alteration of genes which affect the bacterium's ability to sythesize or acylate LPS (lipopolysaccharide)

d. Alteration of genes which affect synthesis of RNA and DNA;

e. Alteration of genes which degrade aberrant periplasmic proteins.

2) Enablement of other bacteria

The Declaration of Dr. Donata Sizemore provides evidence which demonstrates that several other types of bacteria, including *Salmonella*, *E. coli*, and *Listeria*, are able to function as delivery vehicles (see paragraphs 6-24), and that persons of ordinary skill in the art could construct suitable bacterial mutants without undue experimentation.

The Examiner has taken the position that applicant has "admitted" that *Salmonella typhimurium* is an example of a bacteria which is not efficient at delivery of DNA, and referred to Sizemore et al. (Vaccine 15:804-807, 1997) wherein it is stated that *asd* mutants of *Salmonella* will need to be "further engineered" before it will be efficient as a DNA

delivery vehicle. This statement regarding further engineering of *Salmonella* appears in two articles directed to the vaccine community. Because *Salmonella* neither efficiently escapes the vacuole nor carries high copy plasmids, it may not deliver enough plasmid DNA *in vivo* to result in the induction of a strong immune response, but may deliver enough DNA for gene therapy and correction of inborn errors, as disclosed on page 13 of the specification, and further explained as follows.

As potential bacterial carriers were being selected, the inventors focused on two characteristics, (1) escape of the vacuole to the cell cytoplasm and (2) carrying as many copies of the expression plasmid as possible. These characteristics were focused on because of the concern that the harsh environment of the vacuole and nucleases present in the dying bacterium would lead to destruction of many of the copies of the plasmid. As stated in *Science* paper (Science 270:299-302, 1995) there was no formal proof that either escape or high copy plasmids were required. Since *Shigella* both escaped the vacuole and carried high copy plasmids, the initial work was done using that organism. After success with attenuated *Shigella*, experiments were begun using an *asd-Salmonella* strain available in the laboratory. Unfortunately, but not unsurprisingly, the *Salmonella* strain would not grow well while carrying the high-copy pUC plasmid. Reduction of the copy number of the expression plamid resulted in positive *in*

vitro delivery without further engineering the carrier, thus satisfying the objective of the invention of introducing expressible DNA into cells *in vitro*, as described in paragraph 6 of the Declaration of Dr. Sizemore. Thus, it is submitted that the use of *Salmonella* and of other bacteria according to the invention requires no more than routine experimentation.

3) Enablement of target cells

The Declaration of Dr. Sizemore also provides evidence demonstrating that the delivery system can be used in animal cells other than mammalian cells (see paragraphs 27-31 of the Declaration), particularly in avian cells.

In view of the above, applicants respectfully submit that the invention, as presently claimed, is fully enabled. Withdrawal of the § 112, first paragraph rejections is respectfully requested.

Claim 30 was rejected under 35 USC § 112, first paragraph, for insufficient description of *Shigella flexneri* clone 15D. Applicants confirm that the deposit of *Shigella flexneri* clone 15D (ATCC accession no, 55710) was made under the terms of the Budapest Treaty September 6, 1995 and that the deposit will be irrevocably and without restriction or condition released to the public upon the issuance of a patent in the present application. A copy of the receipt for the deposit is enclosed herewith. It is believed that sufficient depository information is contained in the specification.

Claim 44 was rejected under 35 USC § 112, second paragraph, as being indefinite. It was the Examiner's position that the term "functional nucleic acids" was indefinite. The presently pending claims do not contain this term, and therefore the rejection is moot.

All rejections having been addressed, it is believed that this application is in condition for allowance, and Notice to that effect is respectfully requested.

Respectfully submitted,

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